

## **Dinoflagellates cyst assemblage concerning trophic index for eutrophication from major ports along the west coast of India**

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### **Abstract:**

An overview of dinoflagellates cysts assemblage is presented as a trophic index for three monsoon-influenced estuarine and marine ports along the Indian coast. The cyst distribution (including harmful species) showed a trend of highest abundance and species number in highly eutrophicated estuarine (Cochin-south) followed by medium (New-Mangalore-central) and low (Kandla-north) levels of eutrophicated marine ports. The investigation revealed four new species in the region (*Bitectatodinium spongium*, *Gonyaulax elongatum*, *Brigantedinium* sp. and potential harmful species *Blixaea quinquecornis*-cyst similar to planktonic). Autotrophs dominance in the highly productive Cochin and New-Mangalore ports reveals that, in eutrophic systems, heterotrophs need not always be dominant. The indicator taxa (*Polykrikos*, *Protoperidinium*, and *Lingulodinium*) presence in high density indicated a eutrophic system. This study concludes cyst (species numbers/Fisher- $\alpha$  index/indicator species) as potential eutrophication proxies and emphasizes greater harmful-algal-bloom risks in the high trophic-index ports (Cochin and New-Mangalore).

**Keywords:** Dinoflagellate cyst, ports, eutrophication, trophic index, Harmful Algal Blooms.

## **Introduction**

Dinoflagellates are the most dominant and diverse phytoplankton group in freshwater, estuarine, and marine environments and also include potential invaders. Cysts production, as part of many dinoflagellate life cycles has been reported. In some cases, cysts are produced in response to adverse environmental conditions, for example, changes in temperature, salinity, and nutrient fluxes. Once formed, most of the resting cyst settles on the sediment bottom. They remain viable for several years and acts as an essential source of the planktonic population when suitable conditions return (Matsuoka and Fukuyo 2000). Resting stages are referred to as "time capsules," as they preserve biological material for a long time (Ribeiro et al., 2013). In recent years, concerned with the global increase in the occurrence of Harmful Algal Blooms (HABs) in coastal waters, owing to eutrophication (anthropogenic nutrient enrichment; e.g. Anderson et al., 2002), changing climate patterns (eg. Hallegraeff, 2010) and ships ballast water-mediated introduction of non-native species (eg. Smayda, 2007; Casas-Monroy, et al., 2013), the studies on dinoflagellates and its cysts have received importance. As several of the dinoflagellate species are responsible for the occurrence of HABs worldwide, some of which are toxin-producing and act as environmental proxies. At least 90 of these cysts producing species are known to cause harmful blooms (Sournia et al. 1995), and 45 species are considered toxic (Sournia et al. 1995; Smayda et al. 1997; Hallegraeff et al. 2003). Further, dinoflagellate cyst assemblages are also considered as a valuable tool (for the detection and mapping of HABs; Satta et al. 2013) and proxy to determining past environmental variations in the water conditions (such as changes in sea-surface temperature, salinity, turbulence, and eutrophication along with changes in sedimentary conditions; Zonneveld et al. 2012).

A global atlas on cysts distribution studies in the world ocean suggests that a considerable amount of information is available from all the climatic zones of the world oceans except from the Indian sub-continent (Zonneveld et al., 2013). Despite the above-said importance, the information on dinoflagellate cysts distribution from the Indian subcontinent is scarce and are restricted to modern cysts at few locations (D'Costa et al., 2008; D'Silva et al., 2012, 2013; Godhe et al. 2000; Patil 2003; Narale et al., 2013, 2015, Narale and Anil, 2017) as well as from the sediments representing Late Quaternary period from Bay of Bengal (Naidu et al. 2012). Considering India being one of the significant maritime countries and having a vast coastline (~7,500 km), the studies on cysts producing dinoflagellate need detailed investigation at spatial and temporal scales to better understand their population dynamics holistically. Moreover, Indian coasts are characterized with high particulate-load and high species diversity but low cyst abundance. Therefore characterizing the

distributional patterns of dinoflagellate cysts will be beneficial in improving the understanding of HABs and also to study the spatio-temporal change of dinoflagellate cysts in different environments.

Considering human induced alterations over the years, it is presumed that the influence of such alterations (including eutrophication) will be reflected in dinoflagellates and cyst assemblages *per se*. In this study, the information (such as composition and species diversity and richness) on cyst assemblages from three major ports (Cochin – estuarine Port, Mangalore – marine port, and Kandla – marine port experiencing hypersaline condition during non-monsoon) located along the west coast of India was correlated with the trophic status (by comparing with available indices for eutrophication/pollution) for identifying new environmental proxies. The data generated will provide additional information to the growing body of knowledge on dinoflagellates as water quality proxies and biogeography, including harmful and indicator (nutrient enrichment) species for the less studied monsoon influence tropical ports. The sampling in each port was carried out as part of the port biological baseline survey for BAMPI (Ballast water Management Program, India).

## **Materials and methods**

### **Description of the study area**

#### ***Cochin Port***

Cochin port (Kerala, India) is one of the major Indian ports and is situated on a bar-built estuary at the convergence of backwaters. Cochin ports, Ernakulum channel is 5km long and 200 mts wide, while Mattancherry channel is 2.6 km long and 180 m wide (BAMP Monograph series No. 5, Internal publication). Cochin receives 55–65 % of the total annual rainfall during the monsoon season (Menon et al., 2000). The salinity of the backwaters reaches near-zero values due to the heavy precipitation during the peak monsoon period. The maximum tidal range is 1 m in the port regions, whereas the influence of tides is insignificant (Qasim 2003).

For sampling, a total of 23 stations were selected based on activities such as fishing harbor, jetties, wharf, dry docks, creek mouth, and berths (Fig.1 and suppl. Table 1). Sampling was carried out on four occasions representing different seasons, i.e., post-monsoon (October 2011 and November 2012), pre-monsoon (May 2012), and August 2012 (monsoon). The sampling was performed from morning till noon and was spread over 5 to 6 days on each occasion.

#### ***New Mangalore port***

New Mangalore Port (Karnataka) is a modern all-weather on the West Coast of India. New Mangalore ports, side arm channel is 900 mts long and 198 mts wide, Oil berth channel is 1055 m

long and 234 m wide (BAMP Monograph series No. 9, Internal publication). Iron ore pellets and concentrates, petroleum, oil and lubricants (POL) products, granite stones, container cargo, etc. are the major commodities exported from this port. On the other hand, the major imports of the Port are POL products and crude oil, LPG, Coal, Limestone, Finished Fertilizers, Liquid Ammonia, Phosphoric Acid, Other Liquid Chemicals, Containerized cargo, etc.

Sampling was carried out on four occasions representing different seasons, i.e., post-monsoon (November 2011 and December 2012), pre-monsoon (May 2012), and monsoon (September 2012) in New Mangalore Port. In this study, the water and sediment samples were sampled from 19 stations based on port activities (Fig.1 and suppl. Table 2) for dinoflagellate motile-stages and cysts, respectively. Tides ranged from 0.4 to 1.2 m during the study period.

### ***Kandla Port***

Kandla Port, which is located along the western bank of Kandla creek, is operational throughout the year and thereby referred to as an all-weather port. Kandla ports, channel width vary from 200 to 1000 m and total length is around 28 kms (BAMP Monograph series No. 7, internal publication). Various fertilizer-manufacturing industry, industrial-chemical manufacturing units, salt manufacturing units with saltpans rich in brines are located around the Kandla creek (Shirodkar et al. 2010). The port facilitates the extensive traffic of passenger cargo vessels, ore carriers, oil tankers, freighters, fishing boats, and container vessels in Kandla port. In this study, a total of 26 stations were selected based on port activities (Fig.1 and suppl. Table 3), and sampling was done on four occasions in post-monsoon (October 2014 and 2015), monsoon (August 2015), and pre-monsoon (February 2016).

### ***Sampling***

5-L Niskin sampler was used to collect surface and near-bottom water samples. A multiparameter portable instrument (SONDE) was used to measure temperature and salinity at the site. Dissolved oxygen, biological oxygen demand, and chlorophyll-*a* were estimated by following standard procedures (Parsons, 2013). Nutrient concentrations such as phosphate, silicate, nitrite, nitrate, and ammonia were determined using an autoanalyzer (Skalar San++). To evaluate the trophic status of port waters, TRIX was calculated using the equation  $TRIX = (\log_{10} (\text{chl } a \times a\%O_2 \times \text{DIN} \times \text{DIP}) - k)/m$  (Vollenweider et al. 1998). TRIX scores lesser than 3-4 indicate low eutrophication; scores of 4-5 indicate medium eutrophication with; scores of 5-6 indicate high eutrophication, and scores > 6 indicate elevated levels of eutrophication. Trix score was used to assess the relationship with the dinoflagellates cyst. The Vollenweider 1998 TRIX eutrophication score have been successfully

implemented in Mediterranean ports (Massi et al. 2019) and was also employed in other regions, including ports (eg. Rajaneesh et al 2015, Noyel and Desai, 2020). In this study the sampling was conducted continuously over 3-4 days encompassing different tidal regimes and the TRIX score during this sampling period don't vary significantly (for either of the three ports) suggesting negligible influence of the physical forcing.

Van Veen grab was used to collect bottom sediment samples with a 0.04 m<sup>2</sup> grabbing area. In addition, undisturbed sediment cores (20 cm in length PVC corer with an inner diameter of 2.5 cm) through the top windows of the grab, were collected. Cores were sealed with caps, kept in ice in the dark containers. In the laboratory, sediment cores were sectioned at 2 cm interval, mixed well, and stored in airtight plastic bags at 4 °C in the dark until further dinoflagellate cyst analysis.

### ***Dinoflagellate cyst analysis***

Only the top fraction (0–2 cm), which comprises the recent sampled sediments, was processed using the palynological method (Matsuoka and Fukuyo 2000). Sediment samples were sonicated for 10 mins and sieved through 100 and 10 µm mesh-sizes. The residue retained on the 10 µm mesh was acid-treated with 10% HCL acid and 30% HF acid with distilled water washes in between to remove the acid. Next, the sample was sieved through 10 µm mesh size. The residue retained on the mesh was transferred and suspended in 10 mL of distilled water into a vial. Aliquots of 0.25–1 mL of the residual samples were diluted to a total volume of 2.5 mL in a Petri dish (diameter 3.8 cm) and scanned under an inverted microscope (Olympus IX 71) at 100x to 400x magnification. Dinoflagellate cysts were identified based on published literature (Wall and Dale 1968; Sonneman and Hill 1997; Matsuoka and Fukuyo 2000). The cyst abundance was expressed as the number of cysts g<sup>-1</sup> dry sediment after calculating the water content according to the formula given by Matsuoka and Fukuyo (2000).

## **Results**

### ***Environment***

In all the studied ports, the measured environmental parameters revealed a distinct seasonal variation (for details, please see Rath et al. 2018 for New Mangalore port, Rodrigues et al. 2019 for Cochin port, Sathish et al. 2020 for all three ports). However, the seasonal trends between the ports were different (Rath et al. 2018, Rodrigues et al. 2019, Sathish et al. 2020.) The averaged salinity variations were small (30–40 psu) in marine ports (Kandla, New Mangalore), and large (8–38 psu) estuarine ports (Cochin). The low salinity observed in marine and estuarine ports was due to riverine discharge and precipitation during monsoons. Water temperatures in all three ports are relatively

warmer in pre-monsoon and cooler during southwest monsoon (SWM) and post-monsoon. Interestingly, nutrients (NO<sub>3</sub>, PO<sub>4</sub>, and SiO<sub>4</sub>) were more in estuarine than marine port systems. However, the concentrations of nutrients varied between the ports. The nutrient concentrations were higher for Kandla as compared to Cochin and New Mangalore ports (Table 1). Nutrients showed a distinct seasonal variability among the marine ports, but the seasonal trends varied between the ports located. The trophic index (TRIX) revealed that Kandla port (3-4) had a higher state of water quality with low eutrophication level, Mangalore port (4-5) had a good state of water quality with medium eutrophication level, and Cochin port (5-6) had a bad state of water quality with high eutrophication level.

### ***Dinocyst distribution, and diversity***

Overall a total of 40 dinocysts were reported belonging to 17 genera and 2 unidentified dinocysts in the three different port ecosystems (Table 1; Figs. 2 and 3). The autotrophic and heterotrophic dinocyst consists of 20 species each. In addition, a total of 8 potentially HAB species were also recorded in this study. The distribution of dinocyst abundance showed distinct variations between the ports. Dinocyst abundance was highest in Cochin station (1721 cyst<sup>-1</sup>g of DW sediment), followed by Mangalore station (864 cyst<sup>-1</sup>g of DW sediment) and Kandla station (24 cyst<sup>-1</sup>g of DW sediment). This study revealed a high species number in Cochin (35 species) followed by New Mangalore (32 species) and Kandla (15 species) (Fig. 3 b). A total of 7, 8, and 3 species found in Cochin, New Mangalore, and Kandla port are known to be potential HAB species (Table. 2). Of the 8 HAB species, 7 were autotrophic forms, and one was heterotrophic form. The common HAB species found in all ports were *Gonyaulax spinifera* complex, *Lingulodinium polyedra*, and *Protoceratium reticulatum*. In New Mangalore and Cochin port, HAB species found were *Alexandrium tamarense* complex, *Cochlodinium sp*, *Gonyaulax scrippsae*, and *Scrippsiella acuminata* (Table.2). In contrast, HAB species of *Blixaea quinquecornis* was found only in New Mangalore port. The highest HAB dinocyst abundance was recorded in Cochin, followed by New Mangalore and Kandla port. Overall the autotrophic dinoflagellates dominated in Cochin and New Mangalore ports (Fig. 3b). While heterotrophic dinoflagellates were dominated in Kandla port (Fig. 4b). *Pyrophacus steinii* and *Gonyaulax spinifera* complex were the most dominant species in Cochin and New Mangalore ports (Fig. 3a). *Brigantedinium spp* and *Bitectodinium spongium* were the dominant species in the Kandla port (Fig. 3b).

The univariate measures, such as species number, species richness, species diversity, and fisher's index, were also highest in Cochin, followed by New Mangalore and then in Kandla port (Fig. 4a, c, d, e, and f). The ANOVA of dinocyst abundance, species richness, species diversity,

fisher's index, and species number showed a significant variation among the ports. The comparison of the ports revealed a total of 9 species, which were common between the three ports, of which 5 are autotrophic, and four are heterotrophic forms. Of these 9 species, 4 species (*Gonyaulax spinifera* complex, *Lingulodinium polyedra*, *Protoperidinium pentagonum*, and *P. leonis*) were some of the dominant species in each of these ports (Fig. 3). A total 5 species (*Bitectatodinium spongium*, *Brigantedinium* spp., *Gymnodinium microreticulatum*, *Protoperidinium avellana*, and *Protoperidinium denticulatum*) were restricted to only to Kandla port. In comparison, a total 3 species (*Pyrodinium* sp1, *Pyrophacus* sp, and *Protoperidinium* sp1) and 1 species (*Blixaea quinquecornis*) were restricted to Cochin and New Mangalore ports, respectively. Cluster analysis of the dinocyst abundance showed that Cochin and New Mangalore ports were similar to Kandla port (Fig. 6a) as these ports have identical species composition. Cluster analysis of species showed grouping, and at 50% similarity, it formed 2 groups (Fig. 6b). Group, I consist of 7 species represented by rare species with low abundance during the study period (Fig. 6b). While Group II comprised 32 species and was again clustered into two subgroups (IIa, consist of 28 species, and IIb, consisting of 4 species). The species in subgroup IIa, were the dominant species, while species representing subgroup II b are rare species with higher abundance (Fig. 6b).

#### ***Relationship between the community indices and trophic index (TRIX)***

The community indices (abundance, species number, diversity, and richness and fisher's index) versus the TRIX score indicated different scenarios between the ports. In Kandla port, the community indices were the lowest along with low eutrophication levels compared to the other two ports (Fig. 5). In New Mangalore port, the community indices were intermediate along with medium eutrophication levels as compared to the other two ports (Fig. 5). In Cochin port, the community indices were higher along with high eutrophication levels than the other two ports (Fig. 5). In Cochin and New Mangalore Port, the autotrophic cyst abundance was higher with high TRIX scores as compared to Kandla port (Fig. 5a). In Cochin, New Mangalore, and Kandla port, the heterotrophic dinocyst species number and species diversity were higher with a high TRIX score (Fig. 5a). In Cochin and New Mangalore port, the heterotrophic cyst fisher's index was higher with high TRIX scores as compared to Kandla port (Fig. 5d). In Cochin and New Mangalore port, the autotrophic cyst species richness was high at higher TRIX score, and the heterotrophic cyst was high at low TRIX score in Kandla (Fig. 5e).

## Discussion

### *Dinocyst assemblage diversity and distribution*

The present survey on dinoflagellate cyst diversity from three major ports, with different environmental settings along the west coast of India, revealed 42 species. The highest number of cyst types and abundance was recorded in Cochin (estuarine port), followed by New Mangalore port and the least in Kandla (marine but hypersaline) port (Fig. 3a). The prevalence of silty sediment round the year in the Cochin and New Mangalore port could be the reason for the high cyst abundance compared to Kandla ports (Table. 1). Studies suggest that the higher cysts abundances are directly proportional to higher dinoflagellate production in areas with fine-grained sediments (Dale 1983; Anderson et al. 1995; Joyce et al. 2005; Rodrigues et al. 2019). Even considering the reported cyst assemblage from the other major ports Mumbai and JNPT Ports (D'Costa et al. 2008) and Goa (D'Silva et al. 2012) along the west coast of India, cyst abundance and diversity was highest and lowest in Cochin and Kandla port, respectively (Table. 2). These findings suggested that the dinoflagellate cyst abundance and species number showed a decreasing trend with respect to the geographical location of the ports (i.e., from south to north) in the following order: Cochin (southern port) - New Mangalore – Goa – Mumbai - Kandla (northern port). Interestingly, the change in autotrophic and heterotrophic dominance according to the geographical location of the port was also evident. For instance autotrophic and heterotrophic cysts were dominant in southern (Cochin and New Mangalore) and northern (Mumbai/JNPT and Kandla) ports, respectively in the present study. D'Silva et al. (2012) reported heterotrophic dominance along the west coast of India. Still, Rodrigues et al. (2019) observed a decadal change in the dominance from heterotrophic to autotrophic forms in the Cochin Port. Such a paradigm shift might be due to change in environmental settings as the autotrophs and heterotrophs respond directly and indirectly to the environment.

Species composition revealed that though most of the species were recorded in the studied ports, few species with port-specific distribution are considered uncommon species. For instance, the species number of 5, 1, and 3 occurred only in Kandla, New Mangalore, and Cochin Port, respectively (Fig. 3). Before this study, available reports from the major ports (Goa, Mumbai, and Mangalore), suggested the presence of 69 cysts types (Godhe et al. 2000; D'Costa et al. 2008; D'Silva et al. 2012, 2013), and with the present study, due to record of 6 new species records (including one potential HAB species), the total no of cysts types is 75. The four new species include *Bitectatodinium spongium* (autotrophic), *Gonyaulax elongatum* (autotrophic), *Blixaea quinquecornis* (heterotrophic and potential HAB species), *Brigantedinium* spp. (heterotrophic). Among the studied ports, only Cochin port has some cyst data for the year 2000 (D'Silva et al. 2012), and the



comparison indicated that the present study listed 11 more cyst types (including three potentially harmful). Even the density of the dominant species (some *Protoberidinium* spp. and *Gonyaulax spinifera* complex) showed an increasing trend over the last decade (Rodrigues et al. 2019). Nevertheless, the detailed checklist of cyst types and morphological details presented here as part of the port biological baseline survey (PBBS) will be valuable in the studies related to microalgal (dinoflagellate per se) biodiversity and invasion, hereafter.

Documenting cysts of harmful dinoflagellates from the field is considered one of HAB monitoring strategies (Anderson et al., 2012; Ribeiro et al., 2012) as viable cysts can be a precursor for blooms. Given this, the present study lists potential 11 HAB species from the six major ports along the west coast of India (Table 2). Of the 11 species, 9 (*Alexandrium tamarense* complex, *Cochlodinium* sp, *Gonyaulax scrippsae*, *G. spinifera* complex, *Scrippsiella acuminata*, *Protoceratium reticulatum*, *Lingulodinium polyedra*, *Pyrodinium bahamense* and *Blixaea quinquecornis*) potentially HAB species are documented, highlighting the potential HAB risks under favorable conditions. Most of these HAB species are known to produce various toxins (e.g. ichthyotoxins, saxitoxins, and yessotoxins), and also responsible for oxygen depletion and discoloration of water (Table 2). The following dinoflagellates, *Alexandrium tamarense* complex, *Scrippsiella acuminata*, *Gonyaulax spinifera* complex, *Protoceratium reticulatum*, and *Lingulodinium polyedra*, which are known for triggering potentially toxic HABs, and these species were most dominant in the Cochin and New Mangalore ports (Fig. 3). Although no major HABs have been reported during the investigation, the previous HAB instances or shellfish poisoning outbreaks in and around the Cochin and New Mangalore ports have also been documented (Madhu et al., 2011; Padmakumar et al., 2012, 2018). Cysts of saxitoxin producing species *Pyrodinium bahamense* have also been reported from Mumbai port on west coast (Table 3) and Visakhapatnam harbour along the east coast of India (D'Silva et al. 2013). However, to our knowledge reports of its bloom are reported. Recent reports of *Gonyaulax* spp. blooms along the Indian coasts (Padmakumar et al. 2018; Baliarsingh et al. 2018; Kumar et al. 2020), and the dominance of cysts (if any) might pose a concern and needs a vigilant approach as there is a rising trend in the incidences of HAB events in the Indian exclusive economic zone (Padmakumar et al. 2012). Cochin and New Mangalore port are highly at risk to future HABs event compared to Kandla port as most HAB species are dominant in this region (Fig. 3). Findings from Satta et al 2010, suggests *Blixaea quinquecornis* may be a benthic pelicle cyst as far as the surface sediment observation was concerned, and also recorded their apparently viable in deeper sediments (6–7cm). The planktonic form of *B. quinquecornis* have also been the reported in Cochin (Rodrigues et al. 2019) and New Mangalore port from the water

column analysis. Although *B. quinquecornis* were reported in both Cochin and New Mangalore ports in the water column only in surface sediment of the latter was recorded. In this study *B. quinquecornis*, cyst shape observed was similar to that of its vegetative stages. This benthic pellicle cyst stages formed by *B. quinquecornis* need further investigation to study their life cycle. Studies indicated that the dinocysts act as a seed population, which in the dinoflagellates population dynamics play an essential role (Ishikawa and Taniguchi 2000). However, a detailed investigation needs to be commenced to monitor the dinocysts for predicting and preventing HABs events from other port (minor) along the coast of India.

### **Dinoflagellate cyst assemblages from the perspective of water quality**

Several studies reported that dinocysts taxa could be used as indicators of water quality change and anthropogenic impact. To assess the water quality including eutrophication and pollution, indices such as species assemblage composition, species diversity, and species-richness are widely used (Hendey 1977; Wu 1984; Sommer 1995; Tsirtsis and Karydis 1998; Pospelova et al. 2002; Price et al. 2017; 2018). Shannon–Wiener diversity index has been used for pollution index in diatom communities (Hendey 1977), and dinocysts based Fisher's  $\alpha$  and the number of species have been used as indicators of environmental conditions in estuarine systems (Pospelova et al. 2002, 2005). In the present study, the highest values for community indices were found to Cochin, followed by New Mangalore and Kandla port (Fig. 5). The low values in the Kandla port could be attributed to low planktonic biomass, high suspended load, high tidal amplitude, and sandy sediment texture in the region (Table 1). The tropical waters, characterized by low abundance and highly diverse dinoflagellate populations, generally expect high diversity and many species. However, the total number of species recorded is comparable between Cochin and New Mangalore ports and the other geographical regions except for Kandla, where the lowest species were recorded (Table. 4). The comparison of diversity and number of species values with available reports from temperate and tropical regions, including earlier studies from the same region, shows that the value of the present study is much lower than that found in the other areas (Table. 4). The decrease in diversity and number of species of dinocyst taxa can be used as a general indicator of eutrophication and pollution (Pospelova et al., 2002, 2005). Also, the decline in Fisher's  $\alpha$  index and the number of species of dinocysts can also be considered a combined signal of toxic or organic pollution in the coastal regions (Pospelova et al. 2005). Therefore the low values found here could be attributed to the moderate to a high state of eutrophication and pollution in the port region. The values in the present study are comparable to the ranges reported from the coast of the Philippines (Baula et al. 2011, Table 4), and thereby the studied ports. According to the scaling index, as proposed by Pospelova et

al. (2002), and Hendey (1977), Cochin, New Mangalore, and Kandla ports can be categorized as moderately to the highly polluted environment depending on the dinocysts diversity indices. Fisher's  $\alpha$  and the number of species values obtained in Cochin port, New Mangalore, and Kandla port are lower than the values stated by Pospelova et al. (2002, 2005) indicated that the ports are polluted. Further, the scaling done with these proxies is on par with TRIX scores (for Cochin port - Rajaneesh et al. 2015 and New Mangalore port - Rath et al. 2019), pollution load index (for Cochin port; Chakraborty et al. 2014), and metal concentrations (for Cochin port; Martin et al. 2012). From the observation made, it is apparent that the diversity indices of dinocysts form useful environmental proxies.

Additionally, dinocyst taxa composition responses is different for eutrophicated systems (Price et al., 2017). For instance, in regions like Sishili Bay, Yellow Sea-China (Liu et al. 2012), British Columbia-Canada (Radi et al. 2007, Krepakevich and Pospelova 2010), Northeast Pacific margin (Radi and de Vernal 2004), Tokyo Bay-Japan (Matsuoka et al. 2003), and Northern Gulf of Mexico, (Price et al. 2018), the heterotrophic dominance of dinocyst taxa like *Archaeperidinium minutum*, *Brigantedinium spp.*, *Polykrikos kofoidii*, and *Protoperidinium leonis* are known to reflect nutrient availability and changes in eutrophication. Usually, in coastal waters, heterotrophic dinoflagellates increase has referred to indicators for eutrophic environments (Dale et al., 1999; Matsuoka et al. 1999; Dale 2009; Price et al. 2018). In our study, more cysts were observed at medium and high eutrophication levels. Still, instead of heterotrophic autotrophic forms were the dominant (Fig. 5a) suggesting that former need not always be dominant in the eutrophic system, as observed in Cochin and New Mangalore ports. The dominance of autotrophic forms observed for most of the year in Cochin and New Mangalore ports were in contrast with other studied regions in the northern sector along the Indian coast, i.e., Mumbai port (northwest coast), Goa port (central west coast), and Visakhapatnam port (Northeast coast) (D'Costa et al., 2008; D'Silva et al., 2012). This dominance of autotrophic cysts does signify its higher planktonic production (Dale, 1983; Anderson et al., 1995; Joyce et al., 2005) and are reflected in this study. The planktonic dinoflagellate data for Cochin port (Rodrigues et al. 2019) and New Mangalore port (Rath et al. 2018) also showed the prevalence of autotrophs than heterotrophs. Such dominance is because of the changes driven by monsoon events (eg. nutrient-rich freshwater influx, the influence of upwelled water; Verlecar et al. 2006; Harnstrom et al. 2009; Madhu et al., 2010a and 2010b), which fuels phytoplankton production compared to the other studied regions on the northern side. While in the Kandla port, the dominance of the heterotrophic form was observed as they can tolerate broader salinity stress (Shin et al. 2010, 2011). Thus, in Kandla port, the high suspended load, along with the low chlorophyll levels, could be

attributed to heterotrophic dominance (Table. 1). Further, the presence of the relatively high abundance of indicator taxa for eutrophic such as *Pentasparsodinium dalei*, *Lingulodinium polyedra*, *Polykrikos kofoidii/schwartzii*, and the number of *Protoberidinium* species do emphasize that the Cochin, New Mangalore, and Kandla port are eutrophic (Dale et al., 1999; Pospelova et al. 2005; Radi et al. 2007; D'Silva et al. 2013; Price et al. 2018). Furthermore some of the dinocyst (such as *Bitectatodinium spongium*, *Gonyaulax digitalis*, *Gymnodinium microreticulatum*, *Polykrikos kofoidii*, *P. schwartzii*, and *Protoberidinium stellatum*) were relatively morphologically smaller in this study than available reports from other regions (Table 1) suggesting further investigation to delineating influencing factors. Nevertheless, the dominance of the dinocyst (*Protoberidinium leonis*, *Protoberidinium conicum*, *Protoberidinium pentagonum*, *Gonyaulax spinifera* complex, *Lingulodinium polyedra*, and *Pyrophacus steinii*) in the sediments of the complex port ecosystem will add information to a growing body of knowledge on dinoflagellate distribution patterns (Marret and Zonneveld 2003; Zonneveld et al. 2013) but also as an indicator of water quality.

## **Conclusion**

This study provides an overview of dinoflagellate cyst assemblage (including new records and HAB species) with respect to trophic index (TRIX) for eutrophication from the major ports located on the west coast of India. The findings suggested that the dinoflagellate cyst abundance and species number showed a decreasing trend with respect to the geographical location of the ports (i.e., from south to north). Interestingly, the cyst abundance, species number, and the number of HAB species were highest in highly eutrophicated (Cochin) port followed by medium (New Mangalore) and low (Kandla) eutrophicated ports. The dominance of autotrophs in eutrophicated Cochin and New Mangalore port confirms that heterotrophs need not always be dominant and this is in contrast to offshore regions where heterotrophs such as *Protoberidinium* tend to be dominant. However, more data points are required to ascertain heterotrophs dominance in the offshore location along the Indian coasts. Furthermore, the relatively high indicator cyst taxa (*Lingulodinium*, *Polykrikos*, and *Protoberidinium* spp) confirm a eutrophic system. Additionally, the investigation records six new species, including a potential HAB species in the dinocyst checklist from the region. This study demonstrates the utility of dinoflagellate cyst assemblage (species numbers, fisher's  $\alpha$  index, and indicator species) as potential indices for eutrophication in monsoon-influenced ports systems. Cyst assemblages also emphasize greater HAB risks in the high trophic index ports (Cochin and Mangalore) than the low trophic index port (Kandla) under favorable conditions.

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## Reference

- Anderson, DM, Glibert, PM, Burkholder, JM. 2002 Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. *Estuaries*, 25(4), 704-726.
- Anderson DM, Butman B, Fong D et al. 1995 Sources, transport, and nutrient environment of toxic 'red tide' populations in the Western Gulf of Maine. In Gulf of Maine News pp. 6–9. Regional Association for Research on the Gulf of Maine.
- Anderson, D. M., Cembella, A. D., and Hallegraeff, G. M. 2012. Progress in understanding harmful algal blooms: paradigm shifts and new technologies for research, monitoring, and management. *Ann. rev. of mar. sci.*, 4, 143-176.
- Baliarsingh SK, Dwivedi R, Lotliker AA, Jayashankar R, Sahu BK, Srichandan S, Samanta A, Parida C, Srinivasakumar T and Sahu, KC (2018) An ephemeral dinoflagellate bloom during summer season in nearshore water of Puri, East Coast of India; *Ocean Sci. J.* **53** (1) 143–147.
- Baula IU, Azanza RV, Fukuyo Y, Siringan FP 2011 Dinoflagellate cyst composition, abundance and horizontal distribution in Bolinao, Pangasinan, Northern Philippines; *Harmful Algae* **11** 33–44.
- Casas-Monroy, O, Roy, S, Rochon, A 2013 Dinoflagellate cysts in ballast sediments: differences between Canada's east coast, west coast and the Great Lakes. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 23(2), 254-276.
- Chakraborty P, Ramteke D, Chakraborty S, Nath BN 2014 Changes in metal contamination levels in estuarine sediments around India—an assessment; *Mar. Pollut. Bull.* **78** (1) 15–25.
- Dale B 1983 Dinoflagellate resting cysts: 'Benthic plankton' In: Fryxell GA (Ed) *Survival Strategies of the Algae* Cambridge Univ Press Cambridge pp 69–136.
- Dale B 2009 Eutrophication signals in the sedimentary record of dinoflagellate cysts in coastal waters. *J. Sea Res.* **61** (1) 103–113.

- Dale, B, Thorsen, TA, Fjellsa, A, 1999 Dinoflagellate cysts as indicators of cultural eutrophication in the Oslofjord, Norway. *Estuar. Coast. Shelf. Sci.* **48** (3) 371–382.
- D’Silva, M.S., Anil, A.C., D’Costa, P.M., 2011. An overview of dinoflagellate cysts in recent sediments along the west coast of India. *Ind. J. Geo-Mar. Sci.* 40 (5), 697–709.
- D’Silva MS, Anil AC, Borole DV, Nath BN and Singhal RK 2012 Tracking the history of dinoflagellate cyst assemblages in sediments from the west coast of India; *J. Sea Res.* **73** 86–100.
- D’Silva MS, Anil AC and Sawant SS 2013 Dinoflagellate cyst assemblages in recent sediments of Visakhapatnam harbour east coast of India: influence of environmental characteristics; *Mar. Pollut. Bull.* **66** 59-72
- D’Costa PM, Anil AC, Patil JS, Hegde S, D’Silva MS and Chourasia M 2008 Dinoflagellates in a mesotrophic tropical environment influenced by monsoon; *Estuar. Coast. Shelf Sci.* **77** 77–90.
- Godhe A, Karunasagar I and Karlson B 2000 Dinoflagellate cysts in recent marine sediments from SW India; *Bot. Mar.* **43** 39–48.
- Hallegraeff GM, Anderson DM and Cembella AD 2003 *Manual on Harmful Marine Microalgae* UNESCO Publishing Paris: 381–592.
- Harnstrom, K, Karunasagar, I, Godhe, A 2009 Phytoplankton species assemblages and their relationship to hydrographic factors—a study at the old port in Mangalore, coastal Arabian Sea.
- Hendey NI 1977 Species diversity index of some in shore diatom communities and its use in assessing the degree of pollution insult on parts of the north coast of Cornwall; *Nova Hedwigia* **54** 355–378.
- Ishikawa A and Taniguchi A 2000 Vegetative cell and cyst assemblages of armoured dinoflagellates in Onagawa Bay, northeast Japan; *Plankton Biol. Ecol.* **47** (1) 12–22.
- Joyce LB, Pitcher GC, du Randt A and Monteiro PMS 2005 Dinoflagellate cysts from surface sediments of Saldanha Bay, South Africa: an indication of the potential risk of harmful algal blooms; *Harmful Algae* **4** (2) 309–318.
- Krepakevich A and Pospelova V 2010 Tracing the influence of sewage discharge on coastal bays of Southern Vancouver Island (BC, Canada) using sedimentary records of phytoplankton; *Cont. Shelf Res.* **30** (18) 1924-40.
- Kumar PS, Kumaraswami M, Ezhilarasan P, Rao GD, Sivasankar R, Rao VR and Ramu K 2020 Blooming of *Gonyaulax polygramma* along the southeastern Arabian Sea: Influence of upwelling dynamics and anthropogenic activities; *Mar. Pollut. Bull.* **151** 110817.
- Liu D, Shi Y, Di B, Sun Q, Wang Y, Dong Z and Shao H 2012 The impact of different pollution sources on modern dinoflagellate cysts in Sishili Bay Yellow Sea China; *Mar. Micropaleontol.* **84- 85** 1–13.
- Marret F and Zonneveld KAF 2003 Atlas of modern organic-walled dinoflagellate cyst distribution; *Rev. Palaeobot. Palynol.* **125** 1–200.

- Martin GD, George, R, Shaiju, P, Muraleedharan, KR, Nair, SM, Chandramohanakumar, N, 2012. Toxic metals enrichment in the surficial sediments of a eutrophic tropical estuary (Cochin Backwaters, Southwest Coast of India); *Sci. World J.* **1** 972839 2012.
- Matsuoka, K., 1999. Eutrophication process recorded in dinoflagellate cyst assemblages - a case of Yokohama Port, Tokyo Bay, Japan. *Sci. Total Environ.* 231(1), 17-35.
- Matsuoka K and Fukuyo Y 2000 Technical Guide for Modern Dinoflagellate Cyst Study WESTPAC-HAB/WESTPAC/IOC Japan Society for the Promotion of Science 29 pp.
- Matsuoka K, Joyce LB, Kotani Y and Matsuyama Y 2003 Modern dinoflagellate cysts in hypertrophic coastal waters of Tokyo Japan; *J. Plankton Res.* **25** (12) 1461–1470.
- Madhu. NV, Jyothibabu, R, Balachandran, KK, 2010a Monsoon-induced changes in the size-fractionated phytoplankton biomass and production rate in the estuarine and coastal waters of southwest coast of India. *Environ. Monit. Assess.* 166, 521-528.
- Madhu, NV, Balachandran, KK, Martin, GD, Jyothibabu, R, Thottathil, SD, Nair, M, Joseph, T, Kusum, KK, 2010b Short-term variability in water quality and its 28 implications for phytoplankton production in a tropical estuary (Cochin backwaters India). *Environ. Monit. Assess.* 170, 287-300
- Madhu NV, Reny PD, Paul M, Ullas N and Resmi P 2011 Occurrence of red tide caused by *Karenia mikimotoi* (toxic dinoflagellate) in the Southwest coast of India. *Ind. J. Geo-Mar. Sci.* **40** (6) 821–825.
- Massi L, Maselli F, Rossano C, Gambineri S, Chatzinikolaou E, Dailianis T, Arvanitidis C, Nuccio C, Scapini F, Lazzara L 2019 Reflectance spectra classification for the rapid assessment of water ecological quality in Mediterranean ports. *Oceanologia.* 61(4):445-59.
- Menon NN, Balchand AN, Menon NR 2000 Hydrobiology of the Cochin backwater system—a review. *Hydrobiologia* **430** (1–3) 149–183.
- Naidu PD, Patil JS, Narale DD and Anil AC 2012 A first look at the dinoflagellate cysts abundance in the Bay of Bengal: implications on Late Quaternary productivity and climate change. *Curr. Sci.* **202** (3) 495-499.
- Narale DD, Naidu PD, Anil AC and Godad SP 2015 Evolution of productivity and monsoonal dynamics in the eastern Arabian Sea during the past 68ka using dinoflagellate cyst records; *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **435** 193–202.
- Narale DD, Patil JS and Anil AC 2013 Dinoflagellate cyst distribution in recent sediments along the south-east coast of India; *Oceanologia* **55** (4) 979–1003.
- Narale DD and Anil AC 2017 Spatial distribution of dinoflagellates from the tropical coastal waters of the South Andaman India: implications for coastal pollution monitoring; *Mar. Pollut. Bull.* **115** 498–506.
- Noyal V, Desai DV 2020 Spatio-temporal variation in the macrobenthos of Paradip port, east coast of India. *Curr Sci.* 119 (1) 68.

- Patil JS 2003 Diatoms in benthic and pelagic environment In: Studies on ecology of diatoms PhD Thesis University of Goa pp 47–50.
- Padmakumar KB, Thomas LC, Salini TC, Vijayan A, Sudhakar M 2018 Subsurface bloom of dinoflagellate *Gonyaulax polygramma* stein in the shelf waters off mangalore-south eastern arabian sea; Ind. J. Geo-Mar. Sci. **47 (8)** 1658–1664.
- Padmakumar KB, Menon NR and Sanjeevan VN 2012 Is occurrence of harmful algal blooms in the exclusive economic zone of India on the rise? ; Int. J. Oceanography **201**, 2000, Article ID 263946.
- Price AM, Baustian MM, Turner RE, Rabalais NN and Chmura GL 2018 Dinoflagellate cysts track eutrophication in the Northern Gulf of Mexico; Estuar. Coast **41 (5)**1322-36.
- Price AM, Coffin MR, Pospelova V, Latimer JS and Chmura GL 2017 Effect of nutrient pollution on dinoflagellate cyst assemblages across estuaries of the NW Atlantic; Mar. Pollut. Bull. **121 (1-2)** 339-351.
- Pospelova V, Chmura GL, Boothman WS and Latimer JS 2002 Dinoflagellate cyst records and human disturbance in two neighboring estuaries New Bedford Harbour and Apponagansett Bay Massachusetts (USA); Sci. Total Environ. **298 (1)** 81–102.
- Pospelova V, Chmura GL, Boothman WS and Latimer JS 2005 Spatial distribution of modern dinoflagellate cysts in polluted estuarine sediments from Buzzards Bay (Massachusetts USA) embayments; Mar. Ecol. Prog. Ser. **292** 23–40.
- Parsons, TR 2013 *A manual of chemical & biological methods for seawater analysis*. Elsevier.
- Qasim SZ 2003 Indian Estuaries. Allied publishers; pp. 201–256.
- Radi T and de Vernal A 2004 Dinocyst distribution in surface sediments from the northeastern Pacific margin (40-60 °N) in relation to hydrographic conditions productivity and upwelling; Rev. Palaeobot. Palynol. **128 (1–2)** 169–193
- Radi T, Pospelova V, de Vernal A and Barrie JV 2007 Dinoflagellate cysts as indicators of water quality and productivity in British Columbia estuarine environments; Mar. Micropaleontol. **62 (4)** 269–297.
- Rajaneesh KM, Mitbavkar S, Anil AC, Sawant SS 2015 *Synechococcus* as an indicator of trophic status in the Cochin backwaters, west coast of India; Ecol. Indic. **55** 118–130.
- Rath AR, Mitbavkar S and Anil AC 2018 Phytoplankton community structure in relation to environmental factors from the New Mangalore Port waters along the southwest coast of India; Environ. Monit. Assess. **190 (8)** 481.
- Ribeiro, S., Amorim, A., Andersen, T.J., Abrantes, F., Ellegaard, M., 2012. Reconstructing the history of an invasion: the toxic phytoplankton species *Gymnodinium catenatum* in the Northeast Atlantic. Biological Invasions 5, 969–985.
- Ribeiro S, Berge T, Lundholm N and Ellegaard M 2013 Hundred years of environmental change and phytoplankton ecophysiological variability archived in coastal sediment; Plos one **8 (4)** e61184.



- Rodrigues RV, Patil JS, Sathish K and Anil AC 2019 Dinoflagellate planktonic-motile-stage and benthic-cyst assemblages from a monsoon-influenced tropical harbour: Elucidating the role of environmental conditions; *Estuar. Coast. Shelf Sci.* **226** 106253.
- Sathish K, Patil JS and Anil AC 2020 Phytoplankton chlorophyll-breakdown pathway: Implication in ecosystem assessment; *J. Environ. Manage.* **258** 109989.
- Satta CT, Anglès S, Garcés E, Lugliè A, Padedda BM, Sechi N 2010 Dinoflagellate cysts in recent sediments from two semi-enclosed areas of the Western Mediterranean Sea subject to high human impact. *Deep Sea Res. Part II Top.* **57** (3-4) 256-67.
- Satta CT, Anglès S, Lugliè A, Guillén J, Sechi N, Camp J and Garcés E 2013 Studies on dinoflagellate cyst assemblages in two estuarine Mediterranean bays: A useful tool for the discovery and mapping of harmful algal species Original Research Article; *Harmful Algae* **24** 65-79.
- Shin, HH, Mizushima, K, Oh, SJ, Park, JS, Noh, IH, Iwataki, M, Matsuoka, K, Yoon, YH, 2010 Reconstruction of historical nutrient levels in Korean and Japanese coastal areas based on dinoflagellate cyst assemblages. *Mar. Pollut. Bull.* **60** (8) 243–1258.
- Shin, HH, Yoon, YH, Kim, YO, Matsuoka, K, 2011 Dinoflagellate cysts in surface sediments from southern coast of Korea. *Estuar. Coasts* **34** (4) 712–725.
- Shirodkar PV, Pradhan UK, Fernandes D, Haldankar SR and Rao GS 2010 Influence of anthropogenic activities on the existing environmental conditions of Kandla Creek (Gulf of Kutch); *Cur. Sci.* **25** 815-28.
- Smayda TJ 1997 Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea; *Limnol. Oceanogr.* **42** (5 - 2) 1137–1153.
- Smayda, TJ 2007 Reflections on the ballast water dispersal—harmful algal bloom paradigm. *Harmful algae*, 6(4), 601-622.
- Sournia A 1995 Red tide and toxic marine phytoplankton of the world ocean: an inquiry into biodiversity In: Lassus P Arzul G Erard-Le Denn E Gentien P Marcaillou-Le Baut C (eds) *Harmful Marine Algal Blooms* Lavoisier Paris pp 103–112
- Sommer, U., 1995. An experimental test of the intermediate disturbance hypothesis using cultures of marine phytoplankton. *Limnol. Oceanogr.* 40(7), 1271-1277.
- Sonneman JA and Hill DRA 1997 A taxonomic survey of cyst-producing dinoflagellates from recent sediments of Victorian coastal waters Australia; *Bot Mar* **40** (1–6) 149–178
- Tsirsis, G, Karydis, M, 1998 Evaluation of phytoplankton community indices for detecting eutrophic trends in the marine environment. *Environ. Monit. Assess.* **50** (3) 255–269.
- Verlecar, XN, Desai, SR, Sarkar, A, Dalal, SG 2006 Biological indicators in relation to coastal pollution along Karnataka coast, India. *Water research*, 40(17), 3304-3312.
- Vollenweider R, Giovanardi F, Montanari G and Rinaldi A 1998 Characterization of the trophic conditions of marine coastal waters with special reference to the NW Adriatic Sea: proposal for a trophic scale turbidity and generalized 832 water quality index; *Environmetrics* **9** 329–357.

- Wall D and Dale B 1968 Modern dinoflagellate cysts and evolution of the Peridinales; *Micropaleontology* **14** (3) 265–304.
- Wu JT 1984 Phytoplankton as bioindicator for water quality in Taipei; *Bot. Bull. Acad. Sin. (Taipei)* **25** (2) 205–214.
- Zonneveld KA, Marret F, Versteegh G JM, Bogus K, Bonnet S, Bouimetarhan I, Crouch E, de Vernal A, Elshanawany R, Edwards L, Esper O, Forke S, Grøsfjeld K, Henry M, Holzwarth U, Kieft J, Kim S, Ladouceur S, Ledu D, Chen L, Limoges A, Londeix L, Lu S, Mahmoud M S, Marino G, Matsouka K, Matthiessen J, Mildenhall DC, Mudie P, Neil HL, Pospelova V, Qi Y, Radi T, Richerol T, Rochon A, Sangiorgi F, Solignac S, Turon J, Verleye T, Wang Y, Wang Z and Young M 2013 Atlas of modern dinoflagellate cyst distribution based on 2405 data points; *Rev. Palaeobot. Palynol.* **191** 1–197
- Zonneveld KA, Chen L, Elshanawany R, Fischer HW, Hoins M, Ibrahim MI, Pittauerova D and Versteegh GJ 2012 The use of dinoflagellate cysts to separate human-induced from natural variability in the trophic state of the Po-River discharge plume over the last two centuries; *Mar. Pollut. Bull.* **64** 114–132

Table 1. Ranges of environmental parameters recorded from Cochin, New Mangalore, and Kandla ports. \* The TRIX score during the sampling period, which conducted continuously over 3-4 days encompassing different tidal regimes and associated changes, didn't vary significantly for either of the three ports and this strengthen the utility of TRIX index.

Parameters	Ports		
	Kandla	New Mangalore	Cochin
Temperature (°C)	26.5 ± 3.6	27.9 ± 3.6	28.7 ± 1.7
Salinity (psu)	36 ± 6	35.2 ± 0.8	20.8 ± 11.3
Dissolved Oxygen (mg/L)	5.7 ± 0.8	3.4 ± 1.8	4.6 ± 2
Phosphate (µM)	4 ± 1.2	2.6 ± 1.3	3.3 ± 1.3
Silicate (µM)	72.7 ± 37.3	22.1 ± 14.2	37.7 ± 16.7
Ammonia (µM)	5.7 ± 5.9	28.3 ± 10	28.2 ± 12.9
Nitrite (µM)	5.4 ± 2.1	2.8 ± 2	0.9 ± 0.7
Nitrate (µM)	12.6 ± 5.6	4.7 ± 4.2	4.7 ± 3.1
Chl a (µg/L)	0.6 ± 0.3	8.8 ± 9.3	20.6 ± 22.2
TRIX score*	3.5 ± 0.7	4.6 ± 0.9	5.5 ± 0.8
Depth (m)	9-10	7.5-14	9.75-13.7
Sediment Texture (%)	Sandy (55-70%)	Silty- clayey (58-64%)	Silty- clayey (55-67%)

Table 2. List of occurrence of potentially harmful dinoflagellates from three ports with brief description.

Sr. No	Species name	Effect of bloom	Toxin type	Cochin	Mangalore	Kandla
	<b>Autotrophs</b>					
1	<i>Alexandrium tamarense</i> complex		Saxitoxin	+	+	
2	<i>Cochlodinium sp</i>		Possible Ichthyotoxic	+	+	
3	<i>Gonyaulax scrippsae</i>	Discolouration of water and oxygen depletion		+	+	
4	<i>Gonyaulax spinifera</i>		YTX	+	+	+
5	<i>Lingulodinium polyedra</i>		YTX	+	+	+
6	<i>Protoceratium reticulatum</i>		YTX	+	+	+
7	<i>Scrippsiella acuminata</i>	Discolouration of water		+	+	
8	<i>Pyrodinium bahamense</i>		Saxitoxin	+		
	<b>Heterotrophs</b>					
9	<i>Blixaea quinquecornis</i>	Discolouration of water and oxygen depletion			+	

Table 3. Checklist of dinoflagellates cyst types for six major ports located along the west coast of India. Values indicate maximum abundance (cysts g<sup>-1</sup> dry sediment) recorded. The initial d, l, b, and p indicates the diameter, length, breadth, and process length of the dinocyst. Bold species names indicate smaller sized dinocyst.

Species list	D'silva et al 2011			Present study			Present study Size (µm)	Literature Size (µm)
	Mumbai	Goa	Cochin	Kandla	Mangalore	Cochin		
<b>Autotrophs</b>								
<i>Alexandrium affine</i> *	2	2	57					
<i>Alexandrium minutum</i> *	2	8	80					
<i>Alexandrium tamarense complex</i> *	3		37		19	78	d= 45-47	
<i>Alexandrium spp</i>			27					
<i>Bitectatodinium spongium</i>				1			d= 40-50, p= 8- 14	d= 63 p= 10 <sup>1</sup>
<i>Cochlodinium cf. polykrikoides</i> *		9						
<i>Cochlodinium sp</i>		10			19	49	d= 40-50	
<i>Gonyaulax digitalis</i>	11	1	74	2	83	37	d= 30, p= 10	l=60-73 b= 45-63 p= 15-25 <sup>1</sup>
<i>Gonyaulax elongatum</i>					1	49	l= 40-45, b= 30-35, p= 10- 12	l = 40-59 b= 26-42 p= 5-9 <sup>1</sup>
<i>Gonyaulax scrippsae</i> *	45	29	27		42	271	d= 30-35 , p= 10-12	
<i>Gonyaulax spinifera complex</i> *	72	28	148	2	820	2561	d= 35-40, p= 13- 15	l=34-50 b= 34-57 p= 12-17 <sup>1</sup>
<i>Gonyaulax spp</i>		10	107		36	79		
<i>Gymnodinium cf. catenatum</i>	2							
<i>Gymnodinium microreticulatum</i>				1				
<i>Gymnodinium impudicum</i>		8			16	45	d= 30-35	d= 36-62 <sup>1</sup>
<i>Lingulodinium polyedrum</i> *	22	10	44	1	233	565	d= 48-50 p= 10-12	d= 31-54 p= 2-21 <sup>1</sup>
<i>Lingulodinium sp2</i>					8	27	d= 40-50 p= 8- 10	
<i>Pentapharsodinium dalei</i>	8	9	40	1	7	22	d= 30-35 p= 5	d= 19-36 p= 1-8 <sup>1</sup>
<i>Pheopolykrikos hartmannii</i>	4	8			6	176	d= 70-75 p= 10	d= 50-60 p= 7-12 <sup>1</sup>
<i>Protoceratium reticulatum</i> *	25	1	120	4	81	443	d= 40-45 p= 10	d= 38-48 p= 7-14 <sup>1</sup>
<i>Pyrodinium bahamense var compressum</i> *	4						d= 35-40 p= 5-7	
<i>Pyrodinium bahamense</i>						7		

Contd..

Species list	Mumbai	Goa	Cochin	Kandla	Mangalore	Cochin	PS ( $\mu\text{m}$ )	PR ( $\mu\text{m}$ )
<b>Autotrophs</b>								
<i>Pyrophacus steinii</i>	29	17	18		919	1230	d= 65-95 p= 10-15	l=80-110 b= 50-55 p= 8-13 <sup>1</sup>
<i>Pyrophacus sp</i>						28	d= 85 p= 10-15	
<i>Scrippsiella trifida</i>		16						
<i>Scrippsiella acuminata*</i>	19	64	28		17	65	l= 38-40 b=32-35	
<i>Scrippsiella spp.</i>		1			4	208	d= 35	
<b>Heterotrophs</b>								
<i>Blixaea quinquecornis *</i>					17		l= 35-40 b= 30-35 p= 2-5	
<i>Brigantedinium spp.</i>				53			d= 38-40	d= 29-53 <sup>1</sup>
<i>Diplopelta parva</i>		12						
<i>Diplopsalis lenticula *</i>	2							
<i>Lebouraia minuta</i>	34		50					
<i>Polykrikos kofoidii</i>	7	16	74		2	118	l= 68-70 b=38-40 p= 5-10	l = 72-125 b= 48-63 p= 6-11 <sup>1</sup>
<i>Polykrikos schwartzii</i>	4	10	148		22	209	l= 55-60 b=50 p= 5-7	l = 60-72 b= 44-55 p= 8.5-14 <sup>1</sup>
<i>Polykrikos sp</i>		9						
<i>Protoperidinium avellana</i>				1			d= 40-45	
<i>Protoperidinium claudicans</i>	4	14	30		25	229	l= 35-38 b= 30-32 p= 3-5	
<i>Protoperidinium compressum</i>	8	9	43		38	107	l= 85-90 b=70-75	
<i>Protoperidinium conicoides</i>	10			1			d= 40-45	d= 29-49 <sup>1</sup>
<i>Protoperidinium conicum</i>	39	33	82	3	33	278	l= 70-75 b= 75-80 p= 10-12	
<i>Protoperidinium denticulatum</i>		8		1			d= 40-45	
<i>Protoperidinium divaricatum</i>	4	9			1	14	l= 70-80 b= 60-70 p= 10-15	
<i>Protoperidinium latissimum</i>	15	44	85	1	186	498	l= 80-90 b= 75-85	
<i>Protoperidinium leonis</i>	36	47	241	4	371	1115	l= 60-70 b= 45-55	l= 60-80 <sup>1</sup>
<i>Protoperidinium oblongum</i>	49	45	108		154	397	l= 70-75 b= 65-70	
<i>Protoperidinium pentagonum</i>	59		28		344	717	l= 70-95 b= 65-85	
<i>Protoperidinium cf. pentagonum</i>	37	48	103					

Contd..

Species list	Mumbai	Goa	Cochin	Kandla	Mangalore	Cochin	PS (µm)	PR (µm)
<b>Heterotrophs</b>								
<i>Protoperidinium subinerme</i>	10	37	60	1	6	21	l= 60-65 b= 50-55	d= 48-60 <sup>1</sup>
<i>Protoperidinium sp.1</i>	4	8	2			11	l= 55-60 b= 50-55	
<i>Protoperidinium sp.2</i>	2							
<i>Protoperidinium sp.3</i>								
<i>Protoperidinium sp.4</i>		4						
<i>Protoperidinium sp.5</i>		21	37					
<i>Protoperidinium stellatum</i>					40	72	l= 85-90 b= 85-90	l = 92-125 b= 82-126 <sup>1</sup>
<i>Protoperidinium spp.</i>		53	80					
<i>Preperidinium meunieri</i>	6	51	111	0	103	119	d= 55-60 b= 48-50	

<sup>1</sup> <https://www.marum.de/en/Karin-Zonneveld/Modern-Dinocyst-Key.html>

Table 4. Comparison of dinoflagellate cyst species richness and diversity along the Indian coast and other regions.

Region	No. of species	Diversity		Species richness	Pollution based on diversity	Reference
		Shannon-Weiner	Fisher's index			
Kandla Port, West coast of India	15	0.6 – 1.3	0.2 – 2.6	1 - 4	moderate to high	Present study
New Mangalore Port, West coast of India	33	0.6 - 2.2	0.3 - 3	1 - 14	moderate to high	Present study
Cochin Port, West coast of India	35	0.5 - 2.6	0.3 - 2.8	2 - 18	moderate to high	Present study
Cochin Port, West coast of India	29	1.6-2.5	-	-	low to moderate	D'Silva et al., 2011
Mumbai Port, West coast of India	32	1-2.6	-	-	low to moderate	D'Silva et al., 2011
Goa Port, West coast of India	35	0.5-2.5	-	-	moderate to high	D'Silva et al., 2011
Vishakapatnam Port , East coast of India	28	0.4-2.4	-	-	moderate to high	D'Silva et al., 2012
Buzzards bay, Massachusetts, East coast of USA	37	-	3.5-6.4	12 -26	low	Pospelova et al., 2005
Bolinao, Pangasinan, West coast of Philippines	34	0.8-2.5	0.6-3.7	3 - 15	moderate to high	Baula et al., 2011



Figure 1. Location of sampling stations in different ports. a) Kandla port, b) New Mangalore port, and c) Cochin port along the west coast of India. The details of the sampling stations are provided in the Supplementary Tables 1 to 3.

Figure 2 a. Micrographs of HAB dinoflagellates (1) *Alexandrium tamarense* complex, (2) *Cochlodinium* sp, (3) *Gonyaulax scrippsae*, (4) *Gonyaulax spinifera* complex, (5) *Lingulodinium polyedrum*, (6) *Lingulodinium* sp2, (7) *Pyrodinium bahamense*, (8) *Scrippsiella acuminata*, (9) *Blixaea quinquecornis*. Scale bar is 20um.

Figure 2 b. Micrographs of dinoflagellates (1) *Bitectatodinium spongium*, (2) *Gonyaulax digitalis*, (3) *Gonyaulax elongatum*, (4) *Gymnodinium microreticulatum*, (5) *Pentapharsodinium dalei*, (6) *Pheopolykrikos hartmannii*, (7) *Pyrophacus steinii*, (8) *Pyrophacus* sp1, (9) *Scrippsiella* sp, (10) *Brigantedinium* spp., (11) *Polykrikos kofoidii*, (12) *Polykrikos schwartzii*, (13) *Protooperidinium avellana*, (14) *Protooperidinium claudicans*, (15) *Protooperidinium compressum*, (16) *Protooperidinium conicoides*, (17) *Protooperidinium conicum*, (18) *Protooperidinium denticulatum*, (19) *Protooperidinium divaricatum*, (20) *Protooperidinium latissimum*, (21) *Protooperidinium leonis*, (22) *Protooperidinium oblongum*, (23) *Protooperidinium pentagonum*, (24) *Protooperidinium* sp1, (25) *Protooperidinium stellatum*, (26) *Protooperidinium subinermis*, (27) *Preperidinium meunieri*. Scale bar is 20um.

Figure 3. Dinoflagellate cysts abundance (cysts g<sup>-1</sup> dry weight) in the three ports Kandla (K), New Mangalore (M) and Cochin (C). \* indicates potential HAB species. Largest bubble corresponds to 4000 cysts g<sup>-1</sup> dry weight

Figure 4. Variations in dinocyst (a) abundance, (b) autotrophic and heterotrophic forms based on nutrition (c) species-richness, (d) Shannon-Wiener's diversity, (e) Fisher's alpha ( $\alpha$ ) index and (f) Species number from the Kandla, Cochin and New Mangalore ports.

Figure 5. Relationship between trophic index (TRIX) with the total community and their nutrition mode forms (i.e. Autotrophs and Heterotrophs) (a) abundance, (b) species number, (c) species-richness, (d) Fishers index and (e) Shannon-Wiener's diversity of the three ports. For motile-stages and cysts the TRIX scores for surface and near bottom water respectively were used. LE, ME and HE refers to low, medium and high eutrophication, respectively.

Figure 6. Dendrogram of dinoflagellates cysts from sediments with respect to (a) ports and (b) species. Species codes are as follows *Alexandrium tamarense* complex (Ale Tam), *Bitectatodinium spongium* (Bit spo), *Cochlodinium* sp (Coc sp), *Gonyaulax digitalis* (Gon dig), *Gonyaulax elongatum* (Gon elo), *Gonyaulax scrippsae* (Gon scr), *Gonyaulax spinifera* complex (Gon spi), *Gymnodinium microreticulatum* (Gym mic), *Lingulodinium polyedrum* (Lin pol) *Lingulodinium* sp2 (Lin sp), *Pentapharsodinium dalei* (Pen dal), *Pheopolykrikos hartmannii* (Phe har) *Protoceratium reticulatum* (Prot rec), *Pyrodinium bahamense* (Pyr bah) *Pyrophacus steinii*, (Pyr ste), *Pyrophacus* sp1 (Pyr sp), *Scrippsiella acuminata* (Scr acu), *Scrippsiella* sp (Scr sp), , *Brigantedinium* spp. (Bri sp), *Polykrikos kofoidii* (Pol kod), *Polykrikos schwartzii* (Pol sch), *Protooperidinium avellana* (Pro ave), *Protooperidinium claudicans* (Pro cla), *Protooperidinium compressum* (Pro com), *Protooperidinium conicoides* (Pro con) *Protooperidinium conicum* (Pro con), *Protooperidinium denticulatum* (Pro den), *Protooperidinium divaricatum* (Pro div), *Protooperidinium latissimum* (Pro lat), *Protooperidinium leonis* (Pro leo), *Protooperidinium oblongum* (Pro obl), *Protooperidinium pentagonum* (Pro pen) *Protooperidinium* sp1 (Pro sp1), , *Protooperidinium stellatum*, (Pro ste), *Protooperidinium subinermis* (Pro sub), *Preperidinium meunieri* (Pre meu).

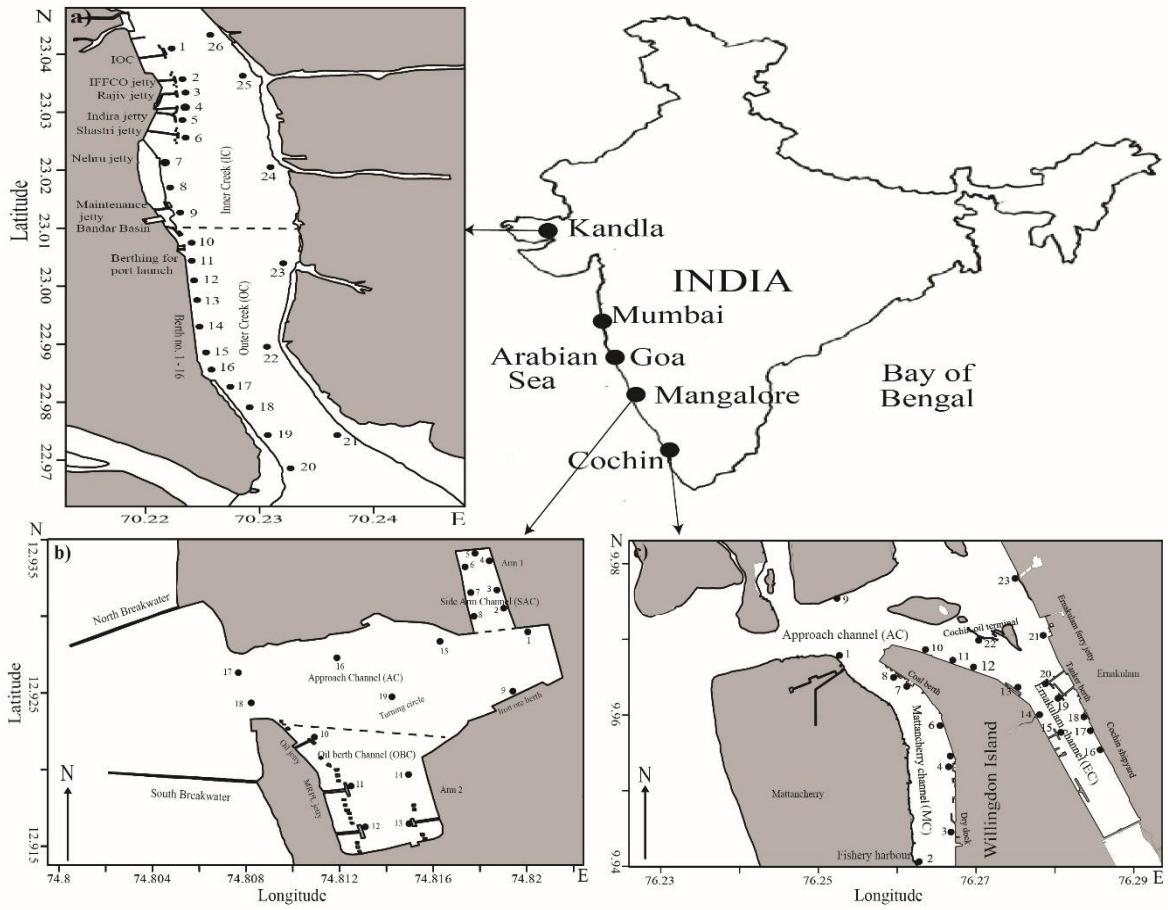


Fig. 1.

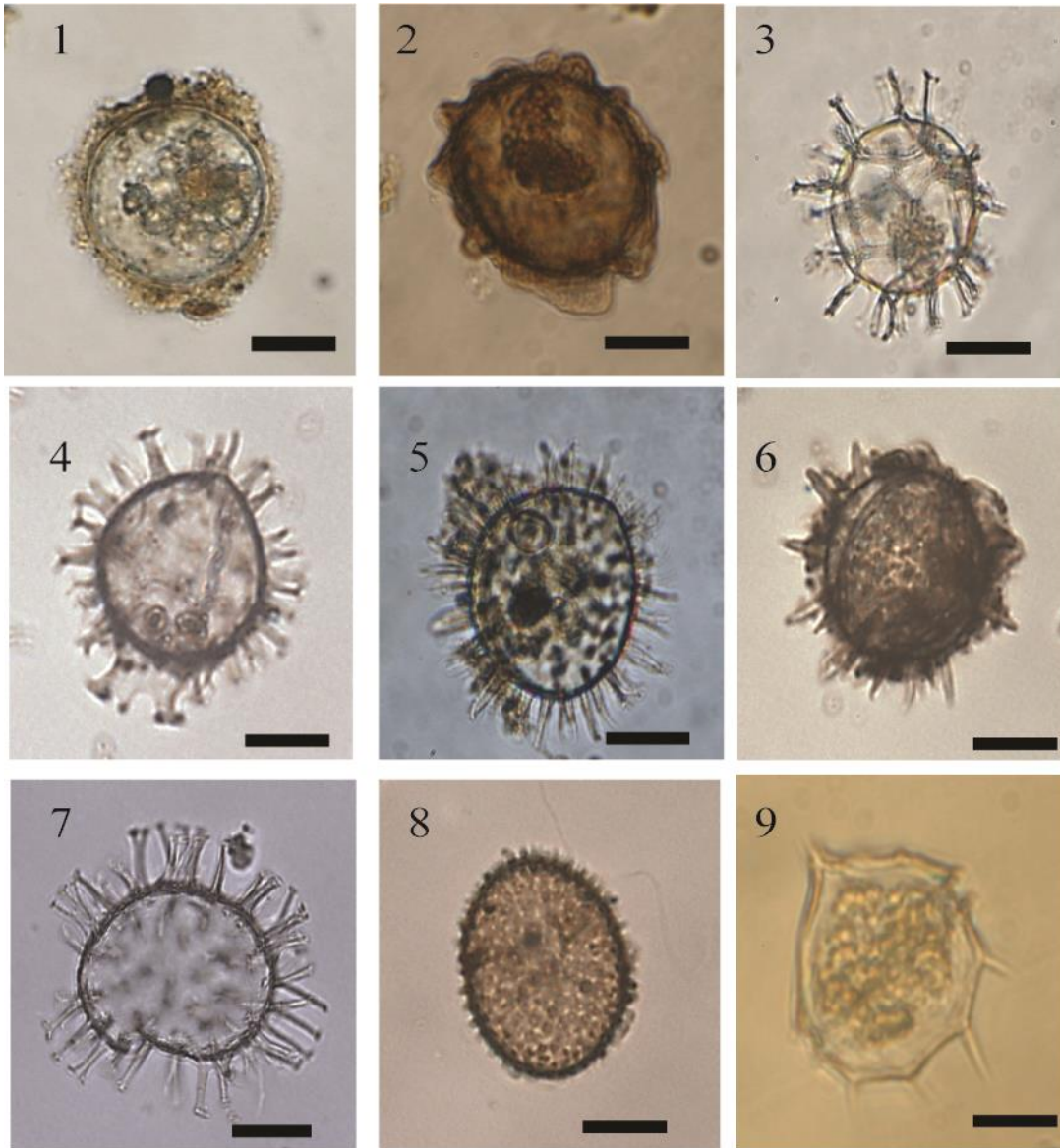


Fig. 2a.

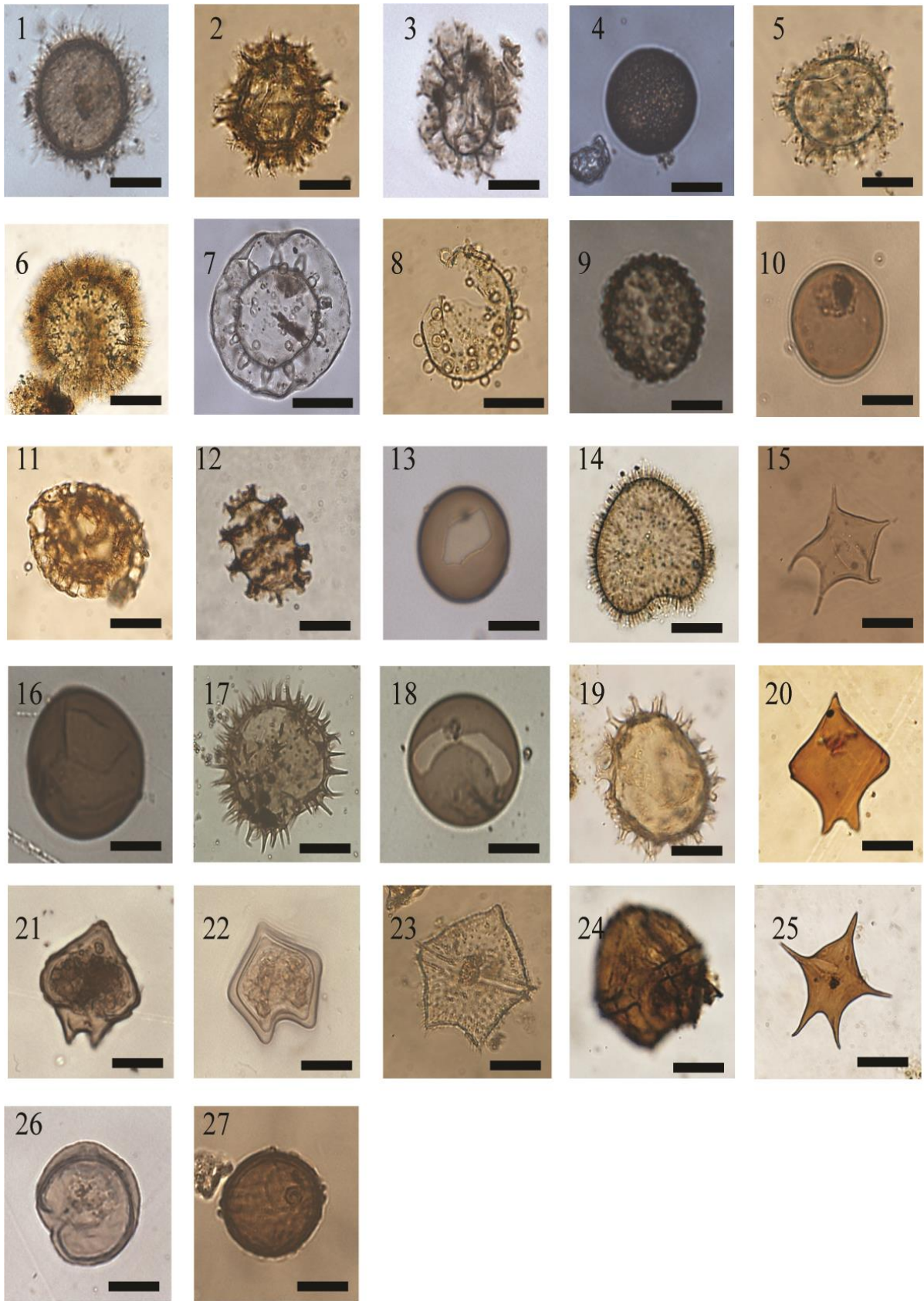


Fig2.b.

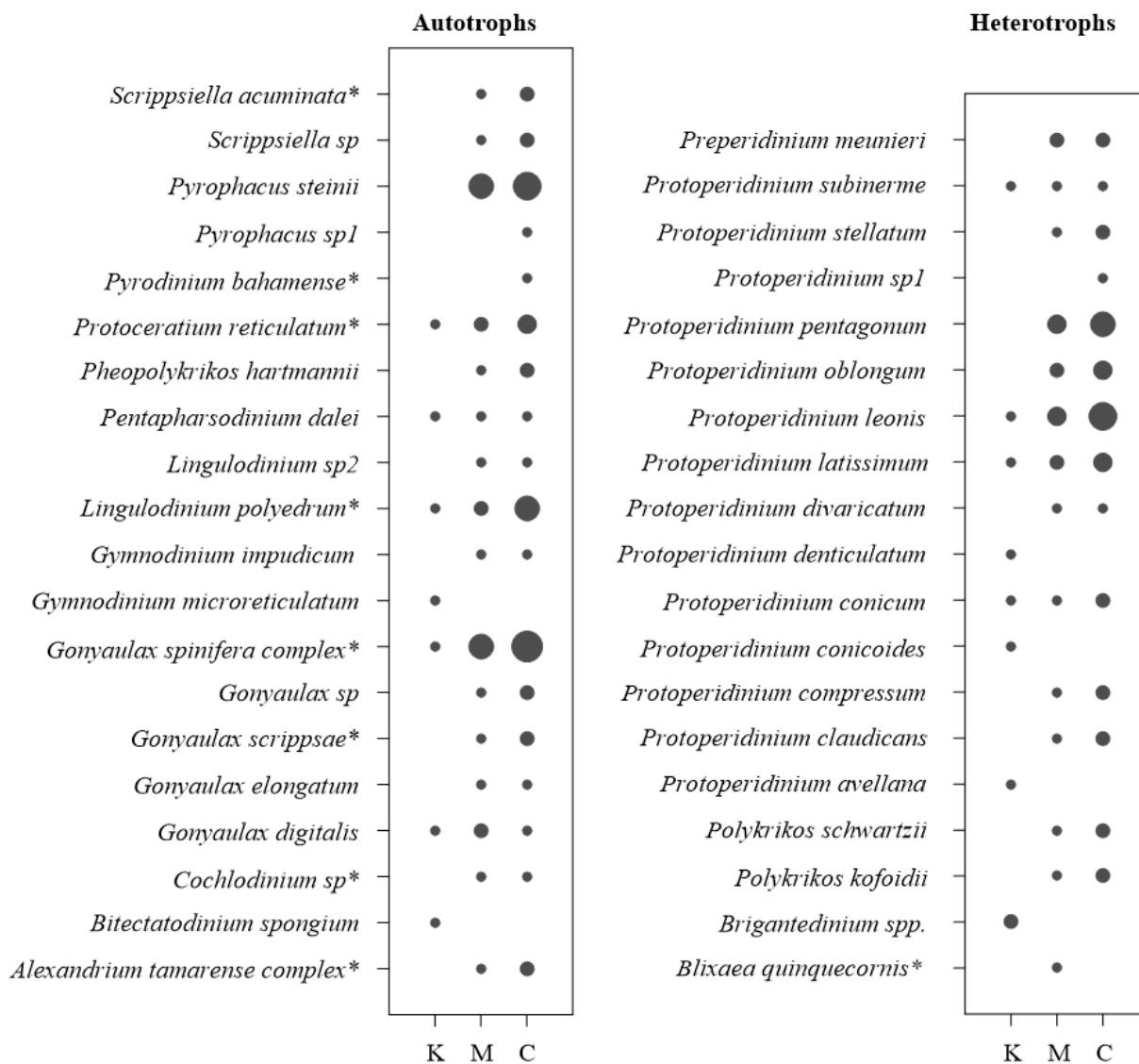


Fig 3.

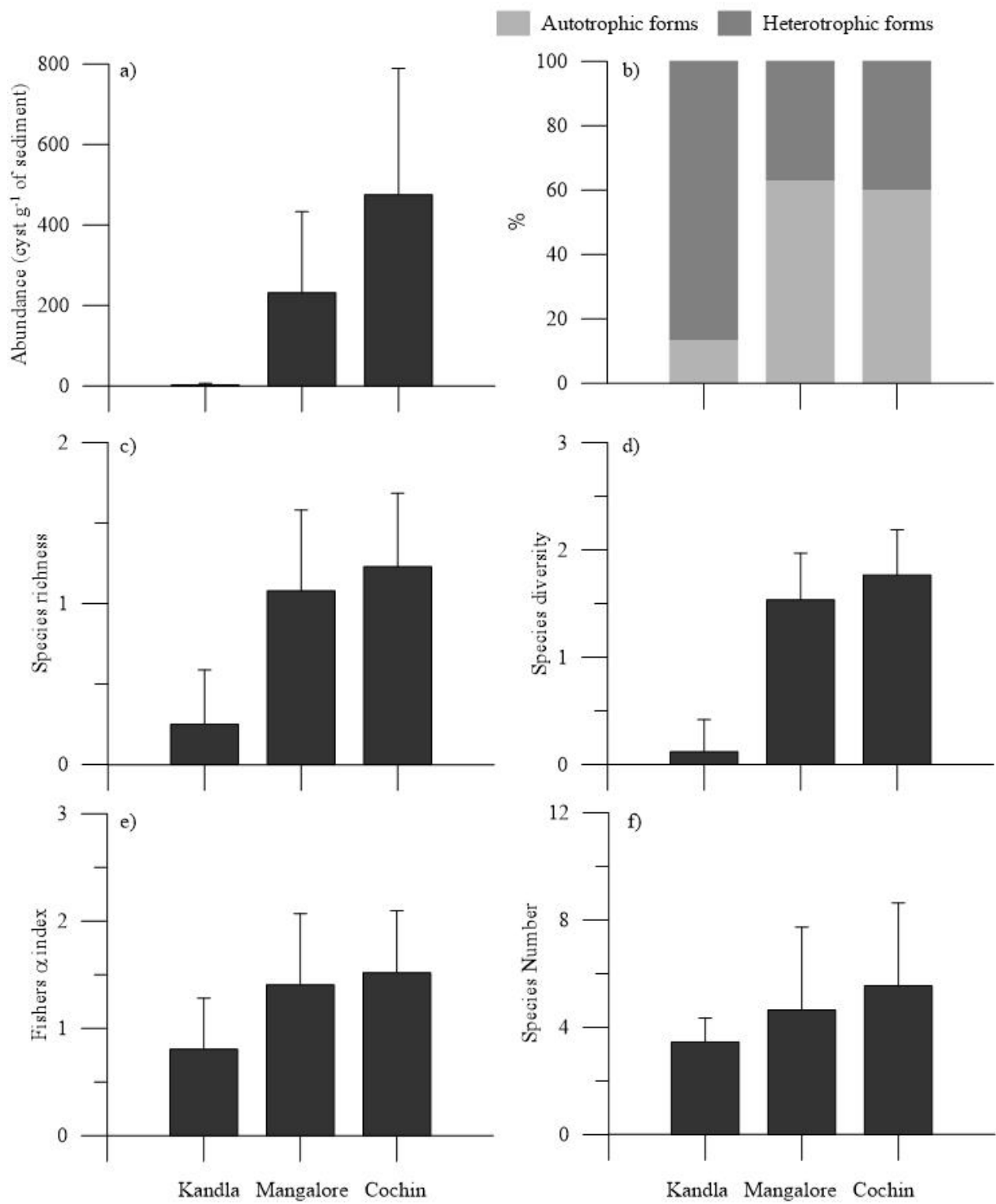


Fig 4.

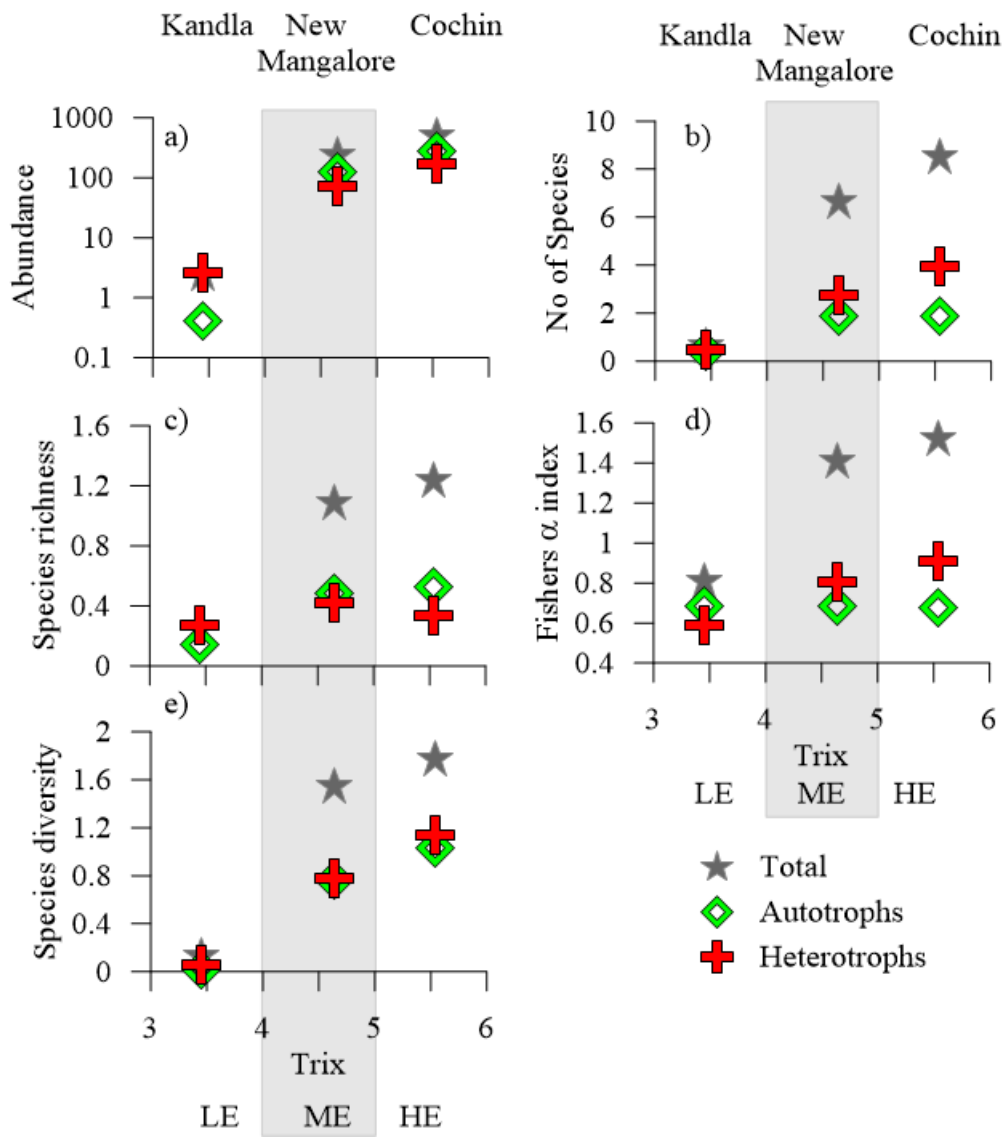


Fig 5.

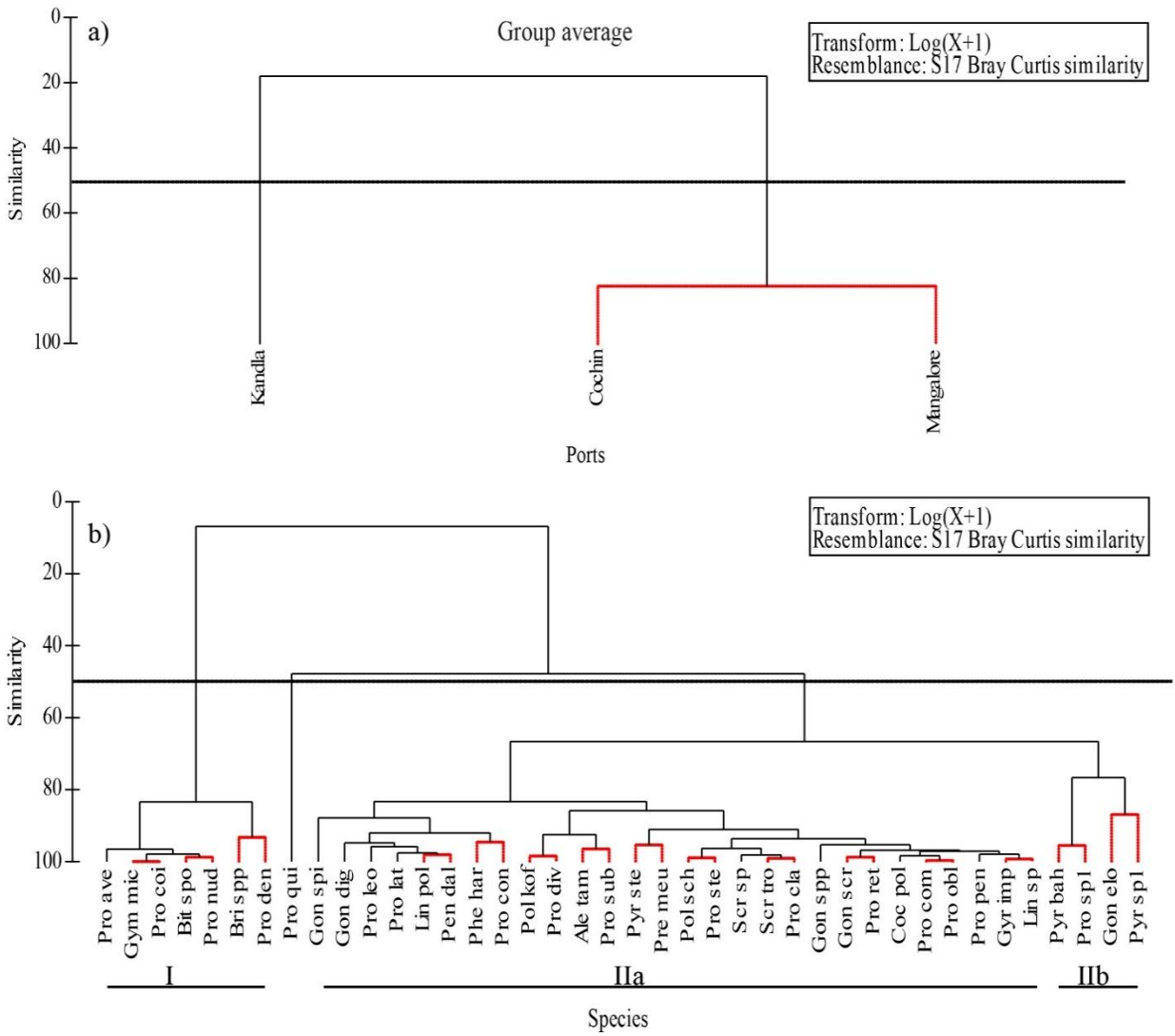


Fig.6



**Suppl. Table no.1.** Details of sampling stations in Cochin harbour.

<b>Stn. No.</b>	<b>Stn. Name</b>	<b>Latitude (°N)</b>	<b>Longitude (°E)</b>
1	Custom Buoy	9.968	76.253
2	Container Terminal	9.975	76.252
3	Fishery Harbour	9.940	76.263
4	L-Jetty/Dry Dock	9.945	76.267
5	South coal berth	9.953	76.267
6	Qua-1	9.954	76.267
7	Qua-4	9.958	76.265
8	North Coal Berth	9.964	76.261
9	Boat Train Pier	9.965	76.260
10	DC Jetty	9.969	76.264
11	Qua-6	9.967	76.267
12	Qua-8	9.966	76.270
13	Qua-10	9.964	76.275
14	Ro-Ro Jetty	9.960	76.278
15	Naval Jetty	9.957	76.281
16	Cochin Shipyard	9.955	76.286
17	Bunker Oil Jetty	9.958	76.285
18	IFP Jetty	9.960	76.284
19	South Tanker Berth	9.962	76.280
20	North Tanker Berth	9.964	76.279
21	Ernakulam Ferry Jetty	9.971	76.279
22	Cochin Oil Terminal	9.970	76.270
23	Ernakulam Creek	9.978	76.275

**Suppl. Table no.2.** Details of sampling stations in New Mangalore port.

<b>Stn. No.</b>	<b>Stn. Name</b>	<b>Latitude (°N)</b>	<b>Longitude (°E)</b>
1	General cargo berth	12.929	74.820
2	General cargo-1	12.930	74.819
3	General cargo-2	12.932	74.819
4	General cargo-3	12.934	74.818
5	Northern return	12.934	74.818
6	General cargo-4	12.933	74.817
7	General cargo-5	12.932	74.817
8	General cargo-6	12.930	74.820
9	Iron ore berth	12.925	74.819
10	POL/Liquefied Gas berth	12.922	74.811
11	Crude/POL berth-1	12.919	74.813
12	Crude/POL berth-2	12.916	74.813
13	Crude/POL berth-3	12.917	74.815
14	Under construction	12.920	74.815
15	Multipurpose berth	12.928	74.816
16	Coal berth	12.927	74.812
17	Channel marker buoy-1	12.926	74.808
18	Channel marker buoy-2	12.924	74.808
19	Turning circle	12.925	74.814

**Suppl. Table no.3.** Details of sampling stations in Kandla port.

<b>Stn. No.</b>	<b>Stn. Name</b>	<b>Latitude</b>	<b>Longitude</b>
1	IOC Jetty	23°02'31.3"N	70°13'20.0"E
2	IFFCO Jetty	23°02'17.0"N	70°13'20.8"E
3	Rajiv Jetty	23°02'04.1"N	70°13'24.3"E
4	Indira Jetty	23°01'54.4"N	70°13'22.9"E
5	Shastri Jetty	23°02'12.6"N	70°13'23.0"E
6	Nehru Jetty	23°02'00.9"N	70°13'23.2"E
7	Local Fishing craft Anchorage-1	23°01'16.3"N	70°13'20.5"E
8	Local Fishing Craft Anchorage-2	23°01'03.0"N	70°13'19.0"E
9	Dry Dock	23°00'45.10"N	70°13'21.6"E
10	Crafts Berthing Jetty	23°00'26.0"N	70°13'24.6"E
11	Cargo Berth-1	23°00'19.7"N	70°13'25.7"E
12	Cargo Berth-3	23°00'13.3"N	70°13'26.4"E
13	Cargo Berth-5	23°00'01.8"N	70°13'26.0"E
14	Cargo Berth-7	22°59'44.7"N	70°13'27.1"E
15	Cargo Berth-9	22°59'32.0"N	70°13'28.3"E
16	Cargo Berth-10	22°59'29.8"N	70°13'28.8"E
17	Cargo Berth-11	22°59'23.7"N	70°13'30.2"E
18	Cargo Berth-12	22°59'17.3"N	70°13'29.5"E
19	Cargo Berth-14	22°59'02.4"N	70°13'35.5"E
20	Cargo Berth-15	22°58'53.3"N	70°13'39.5"E
21	Kandla Creek-1	22°58'56.7"N	70°14'06.6"E
22	Kandla Creek-2	22°59'32.5"N	70°13'55.5"E
23	Kandla Creek-3	23°0'53.28"N	70°13'54.39"E
24	Kandla Creek-4	23°02'10.2"N	70°13'50.5"E
25	Kandla Creek-5	23°2'33.80"N	70°13'35.10"E
26	Kandla Creek-6	23°02'52.0"N	70°13'30.9"E